

IN THE CLAIMS:

Please add new claim 35, cancel claims 19, 27, 28, 30, 33, and 34, and amend claims 17, 18, 20, and 21, as shown below in the detailed listing of all claims which are, or were, in the application.

Claims 1-16 (canceled)

17. (Currently amended) A bioaffinity assay for quantitative determination in a person's sample of free PAPP-A, defined as pregnancy associated plasma protein A (PAPP-A) that is not complexed to a proform of major basic protein (proMBP), wherein an amount of free PAPP-A present in said sample is determined either

i) by exposing said sample to a first binder which binds total PAPP-A and to a second binder which is reactive with the proMBP subunit of the PAPP-A/proMBP-complex ~~binds only PAPP-A complexed to proMBP~~ and detecting total PAPP-A bound to said first binder and detecting PAPP-A complexed to proMBP bound to said second binder in non-competitive sandwich assays, and calculating a difference between measured total PAPP-A and measured PAPP-A complexed to proMBP, or

ii) by a direct bioaffinity assay measuring only free PAPP-A, by making PAPP-A complexed to proMBP non-capable of participating in a bioaffinity reaction in which said sample is exposed to a binder which binds total PAPP-A, by pre-absorbing PAPP-A complexed to proMBP by the steps of

exposing said sample to a first binder which binds to pro-MBP, allowing said proMBP to bind to said first binder, absorbing said first binder onto a solid phase and separating said first binder and said bound proMBP from said sample, exposing said sample, from which proMBP has been separated, to a second binder which binds total PAPP-A, and detecting the bound PAPP-A,

wherein said first binder and said second binder in i) and ii) are both independently either an antibody or antibody fragment.

18. (Currently amended) The assay according to claim 17, wherein free PAPP-A is determined according to alternative i) and two assays are performed, in which one aliquot of the sample is exposed to a first binder which binds total PAPP-A and the total PAPP-A bound to the first binder is detected, and another aliquot of said sample is exposed to a second binder which is reactive with the

~~proMBP subunit of the PAPP-A/proMBP complex binds only PAPP-A~~
~~complexed to proMBP~~ and the PAPP-A complexed to proMBP bound to the second binder is detected, and the amount of free PAPP-A is calculated as a difference between determined total PAPP-A and PAPP-A complexed to proMBP.

19. (Canceled).

20. (Currently amended) The assay according to claim 17 ~~19~~, wherein in alternative i) the first and second binders are capture binders.

21. (Currently amended) The assay according to claim 17 ~~19~~, wherein in alternative i) the first and second binders are labelled binders.

22. (Previously submitted) The assay according to claim 17, wherein free PAPP-A is determined according to alternative i) as one single dual analyte assay where the sample is exposed to a capture binder, which binds total PAPP-A, and to two detecting binders labelled with different labels, so that a first detecting

binder labeled with a first label is directed to an epitope present in any PAPP-A molecule, where a signal of the first label is detected to give total PAPP-A, and a second detecting binder labeled with a second label is directed to an epitope in a proMBP subunit complexed to PAPP-A, where a signal of the second label is detected to give PAPP-A complexed to proMBP.

Claims 23-34 (Canceled).

35. (New) A method for diagnosing persons suffering from an acute coronary syndrome or persons at risk of acute coronary syndrome, comprising

comparing a value of a marker present in a sample derived from said person to a reference value for said marker,

diagnosing whether said person is at risk of acute coronary syndrome based on said comparison,

wherein said marker either consists of free PAPP-A, defined as pregnancy associated plasma protein A (PAPP-A) which is not complexed to a proform of major basic protein (proMBP), as such, or said marker consists of a ratio selected from the group consisting

of free PAPP-A/total PAPP-A, free PAPP-A/PAPP-A complexed to proMBP, and PAPP-A complexed to proMBP/total PAPP-A,

wherein free PAPP-A is determined by a bioaffinity assay method for quantitative determination in a sample of free PAPP-A, either

i) as a calculated difference between measured total PAPP-A and measured PAPP-A complexed to proMBP, either

a) in a method wherein two assay methods are performed, in which one aliquot of the sample is exposed to a first binder which binds total PAPP-A and said total PAPP-A bound to said first binder is detected in a non-competitive sandwich assay, and another aliquot of sample is exposed to a second binder which is reactive with the proMBP subunit of the PAPP-A/proMBP complex and said PAPP-A complexed to proMBP bound to said second binder is detected in a non-competitive sandwich assay, and the amount of free PAPP-A is calculated as a difference between determined total PAPP-A and PAPP-A complexed to proMBP, or

b) in a method wherein free PAPP-A is determined as one single dual analyte assay where the sample is exposed to a capture binder, which binds total PAPP-A, and to two detecting binders labelled with different labels, so that a first detecting binder

labeled with a first label is directed to an epitope present in any PAPP-A molecule, where a signal of the first label is detected to give total PAPP-A, and a second detecting binder labeled with a second label is directed to an epitope in a proMBP subunit complexed to PAPP-A, where a signal of the second label is detected to give PAPP-A complexed to proMBP, or

ii) by a direct bioaffinity assay measuring only free PAPP-A, by making PAPP-A complexed to proMBP non-capable of participating in a bioaffinity reaction in which said sample is exposed to a binder which binds total PAPP-A, by pre-absorbing PAPP-A complexed to proMBP by the steps of

exposing said sample to a first binder which binds to pro-MBP, allowing said proMBP to bind to said first binder, absorbing said first binder onto a solid phase and separating said first binder and said bound proMBP from said sample, exposing said sample, from which proMBP has been separated, to a second binder which binds total PAPP-A, and

detecting the bound PAPP-A, wherein said first binder and said second binder are both independently either an antibody or antibody fragment.